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DNA Flow Cytometry in Gastric Carcinoma: Implication in Patients With Potentially Curative Resection

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Background and Objectives: The relevance of DNA ploidy as a prognostic factor in patients with gastric cancer is controversial. The prognostic significance of DNA ploidy and its relationship to conventional histological grading and staging of the tumor (TNM stage, Lauren, Ming and WHO classification) were evaluated.

Methods: DNA ploidy of the tumor was determined by flow cytometry on archival material from 76 patients who underwent R0, D2 stomach resection.

Results: DNA aneuploidy was found in 39 cases (51%). No significant association between DNA aneuploidy and either patients' sex, pT, pN, type according to Ming or Borrmann and tumor localization was found. The incidence of DNA aneuploidy was significantly lower in tumors of diffuse type according to Lauren, in signet-ring cell or undifferentiated type (WHO), in grade 3/4 tumors, and in patients younger than 50 years. We found no significant difference in survival of patients with DNA aneuploid when compared to DNA diploid tumors, although the prognosis of the patients with lower DNA index (DI < 1.2) tended to be better than that of higher DNA index (DI > 1.2).

Conclusions: DNA ploidy appears to be of limited prognostic value after R0, D2 resection of stomach cancer.

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KEY WORDS: gastric carcinoma; DNA ploidy; prognosis

INTRODUCTION

The incidence of stomach cancer differs in various countries and is steadily declining in the last few decades [1]. However, despite improved endoscopic diagnosis and improvements in surgical treatment, its mortality remains high. Improving the survival rate of patients can be achieved by detection of tumor at an early stage when the tumor is limited to mucosa or submucosa and lymph node metastases are rare. Five-year survival rates of early gastric cancer are >90% [2]. In advanced cancer, only 30–40% of potentially curatively operated patients survive more than 5 years [3,4]. The most important prognostic factor is tumor stage (TNM) at the time of resection [5]. Several other factors can be used to predict survival, including histological type (Lauren), growth type (Ming), macroscopic type (Borrmann), and tumor grade (G) [6]. DNA ploidy recently has been shown to have a prognostic relevance in a large number of solid cancers, and it has been suggested that DNA ploidy can be used as a prognostic factor in gastric cancer [7].

The aim of this study was to investigate the DNA content of gastric cancer cells by flow cytometry using formalin-fixed, paraffin-embedded material, to analyze the relationship between DNA ploidy and known histomorphologic parameters, and to assess its prognostic relevance in patients who underwent a potentially curative (R0) resection of the stomach with D2 lymphadenectomy.

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MATERIALS AND METHODS

The study involved patients with gastric cancer who underwent a potentially curative (R0) resection of the stomach with D2 lymphadenectomy at the Department of Gastroenterologic Surgery (University Medical Center, Ljubljana) during 1988 through 1990. Patients who died within 30 days after resection and those with incomplete follow-up information were not included in the study. Patients with <15 lymph nodes available for pathologic examination were considered inadequately staged and were excluded. There were 79 patients eligible for the study, and these were followed up from 14 to 67 months (median, 47 months).

The tumors were histologically graded and classified according to the WHO [8], Lauren [9], and Ming [10] criteria. The pathologic stage was defined according to the UICC TNM classification [11].

DNA ploidy of the tumors was analyzed by flow cytometry using formalin-fixed, paraffin-embedded material. Cell suspensions were prepared according to a modification of Hedley's method as described by Heiden and coworkers [12]. In brief, two 50-μm sections were cut from the selected paraffin-embedded tissue block, dewaxed with xylene, and rehydrated in a sequence of ethanol solutions. The tissue was then washed and incubated in solution containing 0.1% protease (Protease XXIV, Sigma, St. Louis, MO). The suspension was filtered through a nylon mesh and incubated in a staining solution containing DAPI (4′,6-diamidino-2-phenylindoledihidrochloride, Serva, Heidelberg, Germany). DNA content was measured by PAS II flow cytometer (Partec, Münster, Germany). The cell population was considered diploid when exhibiting a single G0/G1 peak with corresponding G2/M peak in DNA histogram. By definition, DNA index (DI) of diploid cells is 1. Cell populations showing two or more G0/G1 peaks in DNA histograms were classified as aneuploid and the leftmost peak was considered to represent diploid cells [13,14]. DNA index (DI) of tumor cells was calculated by dividing the modal channel number of the aneuploid G0/G1 peak by the modal channel of the diploid one. The quality of DNA histograms was quantified by coefficient of variation (CV) for the diploid G0/G1 peak. Samples showing a CV value of 7 or more were excluded from further analysis.

Statistical analysis of the data was performed by the Chi-square and the Fisher exact tests. Survival distributions were calculated using the method of Kaplan and Meier [15]. The outcomes from different groups of patients were compared by the log-rank test. The Cox regression model was used in multivariate analysis of survival data [16], and $P < 0.05$ was considered statistically significant.

RESULTS

Evaluable DNA histograms with CVs ranging from 2.29 to 6.69 (mean 4.42) were obtained in 76 out of 79 cases. Three cases were excluded from the study because their histograms showed broad peaks with CV > 7.

DNA aneuploidy was identified in 39 cases (51%), whereas 37 cases (49%) were diploid. DNA indexes of aneuploid tumors ranged from 1.11 to 2.29 (mean 1.56). Distribution of various clinicopathological variables and their relation to DNA ploidy are summarized in Table I.

The incidence of DNA aneuploidy did not change significantly in tumors invading the muscularis propria or serosa as compared to those limited to the mucosa and submucosa. The proportion of lymph node positive cases in the DNA aneuploid group was not significantly different from the proportion in the diploid group (59% vs. 54%).

There was no relationship between DNA ploidy and location of the primary tumor, pattern of tumor growth (Ming [10]), or macroscopic type (Borrmann). However, the incidence of DNA aneuploidy was significantly lower in patients younger than 50 years ($P = 0.026$), in tumors of signet-ring cell or undifferentiated histology ($P = 0.020$), in G3/G4 tumors ($P = 0.029$), and in tumors of diffuse type according to Lauren ($P = 0.005$ [9]).

Of the variables shown in Table I, only depth of tumor invasion (pT), lymph node involvement (pN), and pTNM stage significantly correlated with overall survival in univariate analysis. Both pT and pN retained their independent prognostic significance in multivariate analysis with respective relative risk of 2.76 and 1.62 (Figs. 1 and 2).

There was no significant difference in survival between patients with diploid and those with aneuploid tumors (Fig. 3). Survival of patients with lower DNA index (DI < 1.2) tended to be better than that of higher DNA index (DI > 1.2), but the difference did not reach a statistically significant level ($P = 0.09$, Fig. 4). Prognostic relevance of DNA content was also separately tested in several subgroups of tumors, i.e., in early and advanced cancer, in lymph node negative and positive groups, and in tumors of intestinal and diffuse type. In none of these categories did data obtained by flow cytometry provide significant prognostic information.

DISCUSSION

Flow cytometric analysis of archival paraffin-embedded specimens of gastric carcinoma in patients with known outcome allows for retrospective evaluation of relationship among DNA ploidy of tumor cells, histopathological characteristics, and patient survival. In the current study we evaluated these features in a series of patients who underwent potentially curative R0 resection with standard D2 lymphadenectomy, since only in this group of patients can long-term survival be expected, and

TABLE I. Clinicopathological Parameters of 76 Patients With Gastric Carcinoma in Relation to DNA Ploidy

Parameter	N	Aneuploid	%
Sex			
male	43	22	51%
female	33	17	52%
Age (yr)			
<50	20	6	30%
>50	56	33	59%
Location			
upper 1/3	10	6	60%
middle 1/3	34	17	50%
lower 1/3	32	16	50%
pT			
pT1	23	11	48%
pT2	12	7	58%
pT3	41	21	51%
pN			
pN0	33	16	48%
pN1	14	6	43%
pN2	29	17	59%
Pathologic stage (pS)			
pIa	20	10	50%
pIb	7	4	57%
pII	11	4	36%
pIIIa	15	7	47%
pIIIb	23	14	61%
Grade (G)			
G1	6	5	83%
G2	26	16	62%
G3/G4	44	18	41%
Lauren [9]			
intestinal	37	23	62%
diffuse	27	8	30%
mixed	12	8	67%
Ming [10]			
expanding	26	14	54%
infiltrative	36	19	53%
mixed	4	1	25%
undetermined	10	5	50%
WHO [8]			
tubular	41	27	66%
papillary	3	2	67%
mucinous	3	2	67%
signet-ring	21	7	33%
undifferentiated	8	1	13%
Borrmann[a]			
type I + II	23	9	39%
type III + IV	30	19	63%

[a]Borrmann's type not determined in 23 patients with early gastric cancer.

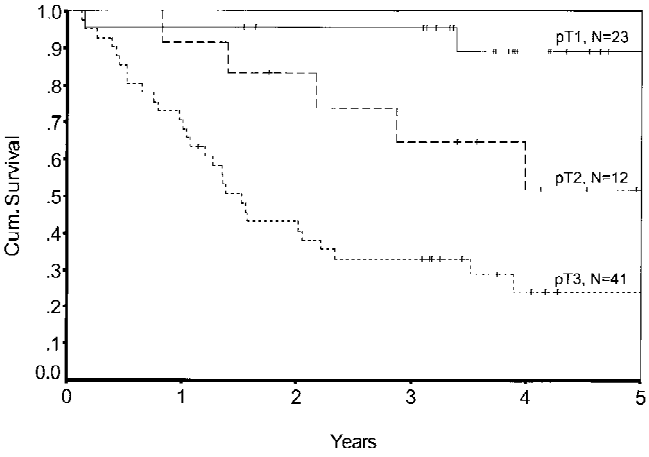

Fig. 1. Effect of depth of tumor infiltration (pT) on survival of patients with gastric carcinoma. The 5-year-survival rate of patients in pT1, pT2, and pT3 category was 89%, 52%, and 24%, respectively. There is a statistically significant difference between these survival curves ($P < 0.001$).

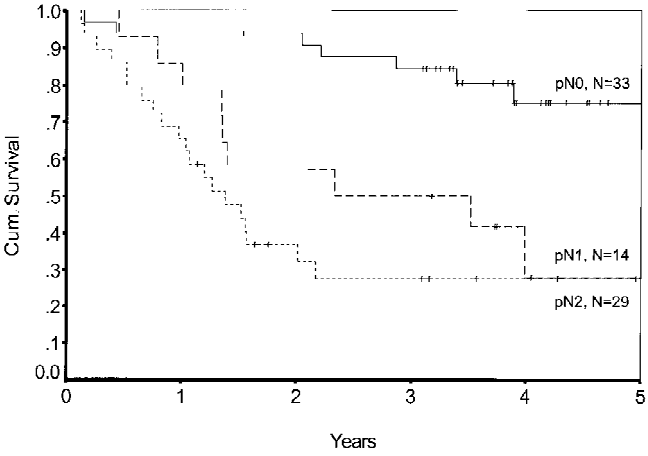

Fig. 2. Influence of lymph node involvement (pN) on survival of patients with gastric carcinoma. The 5-year-survival rate of patients in pN0, pN1, and pN2 category was 75%, 28%, and 27%, respectively. There is a statistically significant difference between survival curves of lymph node negative (pN0) and lymph node positive (pN1 or pN2) patients ($P < 0.001$).

factors influencing prognosis are more important from the clinical point of view [17,18].

The reported incidence of aneuploidy in gastric carcinoma differs widely, from 27–89% [18–20,28]. These discrepant results may be partly due to interlaboratory differences in material (fresh vs. archival), sample preparation, and staining and data acquisition, as well as different classification criteria for ploidy determination [13]. In the present series aneuploidy was detected in 51% of cases, an incidence comparable to that reported in other studies using archival material [18,19,21,25,29].

We found a significant correlation between DNA ploidy and age of the patients, WHO histologic type, histologic grade, and Lauren's type, with diploid DNA pattern being more frequent in patients younger than 50 years, in tumors of signet-ring cell, or undifferentiated histology, in poorly differentiated/undifferentiated (G3/G4) tumors, and in tumors of diffuse type. A higher incidence of aneuploidy in older patients has been reported in several flow cytometric studies of gastric carcinoma [21,29,30], and some investigators also noted its association with a higher degree of differentiation [20,31] and Lauren's intestinal type [18,22]. The latter

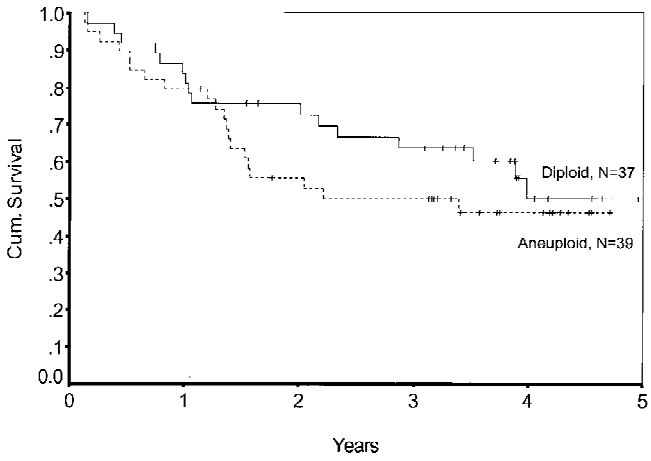

Fig. 3. Influence of DNA ploidy on survival of patients with gastric carcinoma. There is no statistically significant difference between patients with diploid and aneuploid tumors ($P = 0.34$).

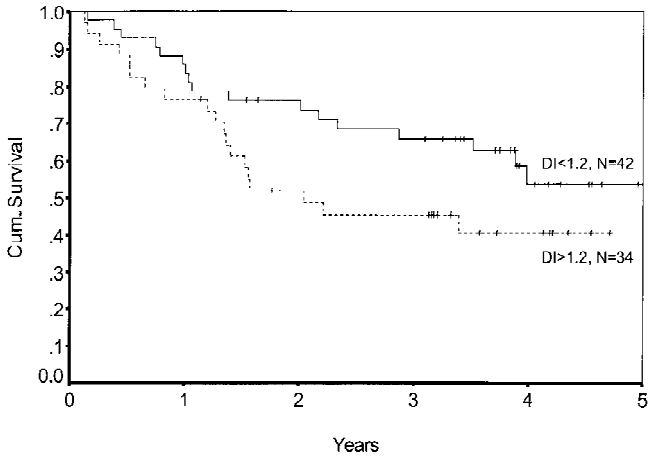

Fig. 4. Influence of DNA index (DI) on survival. Although survival is better for patients with tumors of lower DI (<1.2), the difference between the two survival curves does not reach a statistically significant level ($P = 0.09$).

finding, together with the reported presence of DNA aneuploidy in chronic atrophic gastritis [23,24] and in severe dysplasia of gastric mucosa [32], which are regarded as precursors of intestinal type of gastric adenocarcinoma, supports Lauren's distinction of two different types of gastric carcinoma, i.e., intestinal and diffuse.

Whereas in our analysis as well as in some others [21,25,29,31], no association between DNA ploidy and tumor localization was observed, a higher incidence of DNA aneuploidy was found in proximally located tumors in several studies [22,26,33]. It has been shown that aneuploidy is especially common in tumors located on the cardia [22,26,33]. Since no such tumors were included in the current series, the lack of correlation between tumor site and ploidy is not unexpected. We found no significant difference in percentage of aneuploid cases between early and advanced cancer, which is in accordance with the majority of findings from other studies [20,22,30].

Some authors [21,22,33] observed a higher incidence of lymph node involvement in aneuploid tumors compared to diploid ones. On the basis of these studies, it has been suggested that in patients with diploid tumors, lymphadenectomy can be less extensive than in patients with aneuploid tumors [34]. Our results, as well as those from others [20,31], do not support such a view, since no difference in lymph node involvement was found between patients with diploid and those with aneuploid tumors.

Prognostic relevance of DNA ploidy in gastric cancer appears controversial. Some authors validated its significance in univariate [22,25] and in multivariate [29,35] analyses, but others could not confirm these findings [19–21,27,28,30,31]. With regard to patients who underwent potentially curative surgery, data are similarly inconsistent: DNA ploidy has been found to correlate with survival in some studies [24,29], but not in others, including ours [19,21,27].

The majority of studies that found DNA ploidy of gastric carcinoma to be of prognostic value included large proportions of tumors in the advanced stage [34,35]. Therefore, it seems that the prognostic impact of DNA ploidy is strongest in these tumors. Indeed, results from some studies indicate that ploidy affects survival only in advanced gastric carcinoma [25,30]. Thus, the fact that stage I tumors comprise a relatively large proportion of the present series may be responsible for our failure to find an association between DNA ploidy and prognosis.

In occasional studies a prognostic influence of DNA ploidy was shown only in the intestinal and not in the diffuse type of gastric carcinoma [18,22]. Although we were not able to confirm this, our finding of different ploidy pattern suggests that aneuploidy may have different biological significance and different prognostic implication in these two tumor types.

Most investigations on prognostic value of DNA content in gastric carcinoma are based on comparisons between diploid and aneuploid tumors. However, some studies indicate that aneuploid tumors with higher DI carry a worse prognosis than those with lower DI [35]. In fact, some authors obtained better prognostication when dividing tumors according to their DI [36]. A similar tendency was observed in our series: difference in survival was more evident when comparing tumors with DI > 1.2 and those with DI < 1.2, although it did not reach statistical significance.

CONCLUSIONS

It seems from results of our study that determination of DNA ploidy in gastric adenocarcinoma has little prognostic value in patients in whom a potentially curative

(R0) resection with D2 lymphadenectomy has been performed and whose prognosis is mainly determined by the TNM stage. DNA aneuploidy seems to be the result of tumor progression and becomes an important prognostic factor in stages when the disease is already beyond the reach of current surgical treatment. Flow cytometric determination of DNA content enables observation of changes in cellular DNA during the progression of tumorous growth, but it does not perceive subtle molecular changes that determine the biologic aggressiveness of the tumor.

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